Using multinomial logistic regression method to investigate the farm-level antimicrobial resistance in *Salmonella* in swine

Farzan, A.[(1)]*, Friendship, R.[(1)], Dewey, C.[(1)], Funk, J.[(2)], Poppe, C.[(3)]

[(1)] Department of Population Medicine, University of Guelph, Guelph ON Canada
[(2)] National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI USA
[(3)] Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph ON Canada

Abstract

The objective of this study was to investigate the farm-level risk factors associated with antimicrobial resistance of *Salmonella* spp. on Ontario swine farms. Fecal samples were collected on 80 swine farms and tested for *Salmonella* and antimicrobial resistance. The farms were classified into three groups including *Salmonella*-negative farms (Group 1), *Salmonella*-positive farms without antimicrobial resistance (Group 2), and *Salmonella*-positive farms with antimicrobial resistance (Group 3). No risk factor was associated with the Group 2 classification. The odds of a farm being in Group 3 was 8.4 times higher if pelleted feed was used compared to mash feed ($P = 0.02$). Farms that were grower-finisher operations compared to farrow to finisher operation were 5.2 times more likely to be in Group 3 ($P = 0.007$). The larger farms were more likely to be in Group 3 ($P = 0.035$). There was no evidence of interaction at the level of $P < 0.05$. These findings indicate that the multinomial regression approach is of importance for investigating the risk factors and taking the appropriate approach to each group of farms if a control program were to be implemented or an intervention applied.

Introduction

Salmonella spp continue to be important foodborne pathogens (Mead et al., 1999), particularly serovars demonstrating multiple antimicrobial resistance (Threlfal et al., 2003). Pork products contaminated with *Salmonella* are an important source of *Salmonella* infection in humans. Mutidrug resistant *Salmonella* serovars are associated with increased hospitalization, mortality and consequent economic cost (Travers et al., 2002) and these serovars are emerging as a concern for the global pork industry. Therefore, epidemiological studies need to be conducted in order to gain a better understanding of risk factors for antimicrobial resistance. The objective of this study was to investigate the farm-level risk factors associated with antimicrobial resistance of *Salmonella* spp. on Ontario swine farms.

Materials and methods

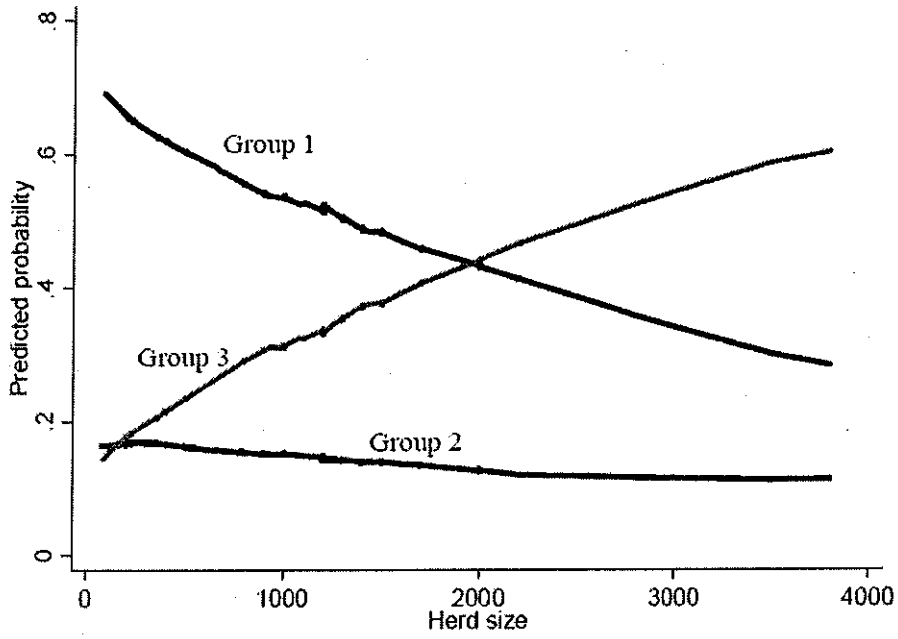
Fecal samples were collected on 80 swine farms and cultured for *Salmonella* (D'Aoust, 1998) and the isolates were tested for antimicrobial resistance (CLSI, 2004 and 2005). Information regarding pig flow, in-feed and water antimicrobial usage, herd size, type of operation, and feed form were collected. A multinomial logistic regression modeling method was used to investigate the risk factors associated with both *Salmonella*-positive farms with antimicrobial resistance and those risk factors associated with *Salmonella*-positive farms without antimicrobial resistance (Dohoo et al., 2003){Dohoo, Wayne, et al. 2003 #2060}.

Results

The eighty farms were classified into three groups including 43 *Salmonella*-negative farms (Group 1), 12 *Salmonella*-positive farms with no antimicrobial resistance (Group 2), and 25 *Salmonella*-positive farms

with antimicrobial resistance (Group 3). In multinomial logistic regression modeling, the association between the risk factors and outcome in Group 2 and Group 3 were compared with Group 1 as the base outcome. No risk factor was associated with the Group 2 classification. The odds of a farm being in Group 3 was 8.4 (95% CI 1.4, 49.8) times higher if pelleted feed was used compared to mash feed (P = 0.02). Farms that were grower-finisher operations compared to farrow to finisher operation were more likely to be in Group 3 (P = 0.007). The shape of relationship between herd size and predicted probability of being in the three groups is shown in Figure. The larger farms were more likely to be in Group 3 (P= 0.035).

Figure 1: The relationship between herd size and probability of being in one of three groups* of swine farms



* Group 1: *Salmonella*-negative; Group 2: *Salmonella*-positive without AMR; Group 3: *Salmonella*-positive with AMR

Discussion

The objective of this study was to investigate the associated risk factors for antimicrobial resistance in *Salmonella*. In-feed and water antimicrobial usage was not associated with *Salmonella* shedding either susceptible (Group 2) or resistant (Group 2) isolates on swine farms. However, the data on drug use in this study was colleted prior to the fecal collection and the drug use policy may have changed on certain farms. Commingling pigs from different sources is more likely on grower-finisher operations than on farrow-finisher farms and this may explain the higher risk of resistant *Salmonella* on the grow-finisher farms. Pelleted feed has been reported before as a common risk factor for increasing *Salmonella* occurrence in swine (Lo Fo Wong et al., 2004; Kranker et al., 2001; von Altrock et al., 2000). However, in the current study, pelleted feed did not appear to be a risk factor for farms testing positive for *Salmonella* with no AMR (Group 2) although it had a significant effect on increasing the probability of classifying a farm as multidrug resistant *Salmonella* group (Group 3). Since antimicrobial resistance was associated with serovar, the impact of feed related factors on occurrence of *Salmonella* might be serovar-dependent. This possibility warrants further studies. The current study is the first study on risk factors for *Salmonella* in pigs using multinomial regression analyzing in that the outcome has been refined into three different

categories. This is of importance for investigating the risk factors and taking the appropriate approach to each group of farms if a control program were to be implemented or an intervention applied.

Acknowledgements

We would like to acknowledge the Public Health Agency of Canada, the Ontario Ministry of Agriculture, Food and Rural Affairs, Ontario Pork, the Canadian Research Institute for Food Safety, and the University of Guelph – OMAFRA Animal Research Program for financial and technical support.

References

CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Information Supplement. Updated tables for the NCCLS antimicrobial susceptibility testing standard M31-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2004.

CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement M100-S15. Wayne, PA: Clinical and Laboratory Standards Institute, 2005, pp. 25, 102–106.

D'Aoust J.Y., U. Purvis 1998. Isolation and identification of *Salmonella* from foods, HPB Method MFHPB-20. Research Division, Health Protection Branch, Public Health Agency of Canada.

Kranker, S., J. Dahl, A. Wingstrand. 2001. Bacteriological and serological examination and risk factor analysis of *Salmonella* occurrence in sow herds, including risk factors for high *Salmonella* seroprevalence in receiver finishing herds. Berl Munch Tierarztl Wochenschr 114: 350-2.

Lo Fo Wong, D.M., J. Dahl, H. Stege, P.J. van der Wolf, L. Leontides, A. von Altrock, B.M. Thorberg. 2004. Herd-level risk factors for subclinical *Salmonella* infection in European finishing-pig herds. *Prev Vet Med* 62: 253-66.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg Infect Dis 5:607-25.

Threlfall, E. J., C. J. Teale, R. H. Davies, L. R. Ward, J. A. Skinner, A. Graham, C. Cassar, and K. Speed. 2003. A comparison of antimicrobial susceptibilities in nontyphoidal salmonellas from humans and food animals in England and Wales in 2000. Microb Drug Resist: 9:183-9.

Travers, K., M. Barza. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clin Infect Dis* 34: Suppl 3, S131-4.

von Altrock, A., A. Schutte, G. Hildebrandt. 2000. [Results of the German investigation in the EU Project "*Salmonella* in Pork (Salinpork)"--2. Investigations in a slaughterhouse]. Berl Munch Tierarztl Wochenschr 113: 225-33.

differences in sampling strategy and culture methods. Yet, it indicates that the antimicrobial resistance in swine is dynamic and that monitoring over time may be useful to detect changes in antimicrobial resistance patterns on swine farms. Among the three groups of farms which defined in the current study, the Group 3 is of particular concern from a public health standpoint and pig production and warrants attention. Further studies need to be conducted to compare the risk factors that distinguish these three groups of farms.

Acknowledgements

We would like to thank the Public Health Agency of Canada, the Ontario Ministry of Agriculture, Food and Rural Affairs, Ontario Pork, the Canadian Research Institute for Food Safety (CRIFS) for the financial and technical support. We also thank the research technicians, and producers who participated in the project.

References

Berends, B.R., F. Van Knapen, D.A. Mossel, et al., 1998. Impact on human health of *Salmonella* spp. on pork in The Netherlands and the anticipated effects of some currently proposed control strategies. Int J Food Microbiol. 44:219-29.

CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Information Supplement. Updated tables for the NCCLS antimicrobial susceptibility testing standard M31-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2004.

CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement M100-S15. Wayne, PA: Clinical and Laboratory Standards Institute, 2005, pp. 25, 102–106.

Dohoo, I., W. Martin, H. Stryhn, 2003. Veterinary Epidemiologic Research. pp. 373-389. AVC Inc., University of Prince Edward Island, Canada

Poppe C, Ayroud M, Ollis G, et al. Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin, and the environment of animal production in Canada, 1994-1997. Microb Drug Resist 2001; 7:197-212

Threlfall, E. J., C. J. Teale, R. H. Davies, L. R. Ward, J. A. Skinner, A. Graham, C. Cassar, and K. Speed. 2003. A comparison of antimicrobial susceptibilities in nontyphoidal salmonellas from humans and food animals in England and Wales in 2000. Microb Drug Resist: 9:183-9.

Wegener, H.C., Hald, T., Lo Fo Wong, D., Madsen, M., Korsgaard, H., Bager, F., Gerner-Smidt, P., Mølbak, K. 2003. *Salmonella* control programs in Denmark. Microb Drug Resist: 9:183-9.